

# An ELISA for studying Ebola Virus / VLP release

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# Introduction/Agenda

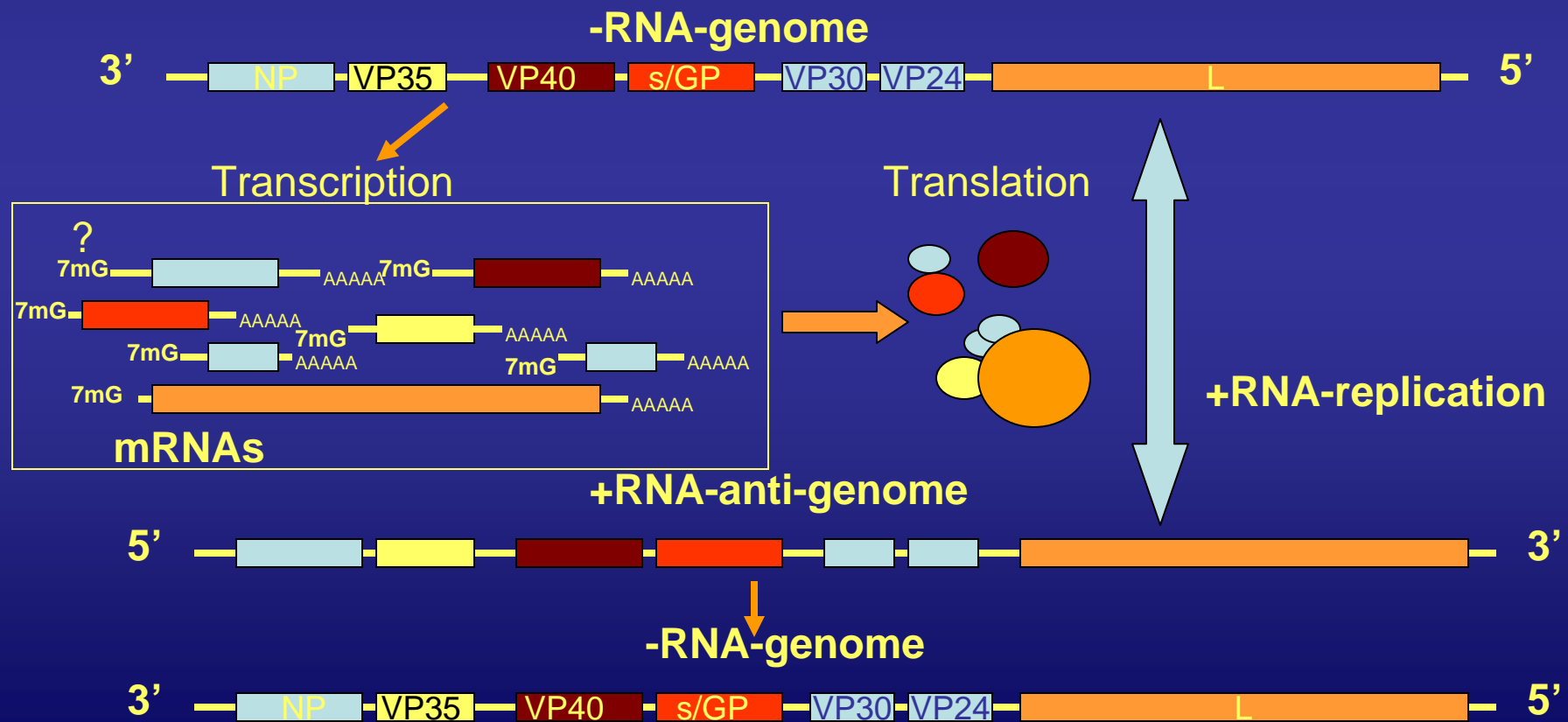
- Filovirus Background
  - Replication
  - Structure
  - VP40
- ELISA setup and optimization
- Effects of Ebola proteins on VLP release
- Effects of VP40 mutants on VLP release
- Therapeutic compound screening

# Filoviruses

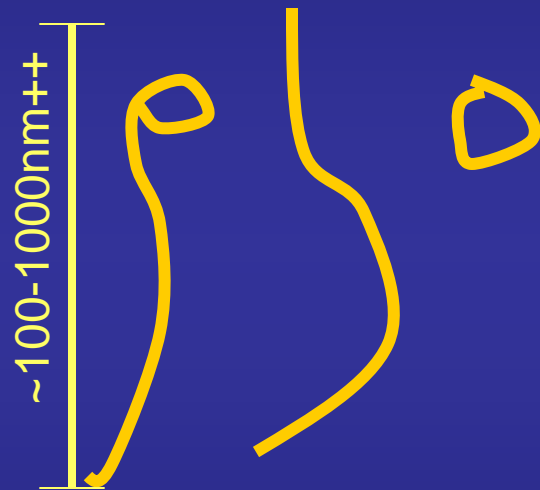
- *Mononegavirales, Filoviridae*-2 species in family (Marburg and Ebola)
- Both clinically manifest as a rapidly progressive hemorrhagic fever with high mortality rates
- Marburg was weaponized and stockpiled by the USSR
- Sporadic outbreaks have been on the rise in Africa over the past several years
- Unknown Reservoir

# Viral Replication

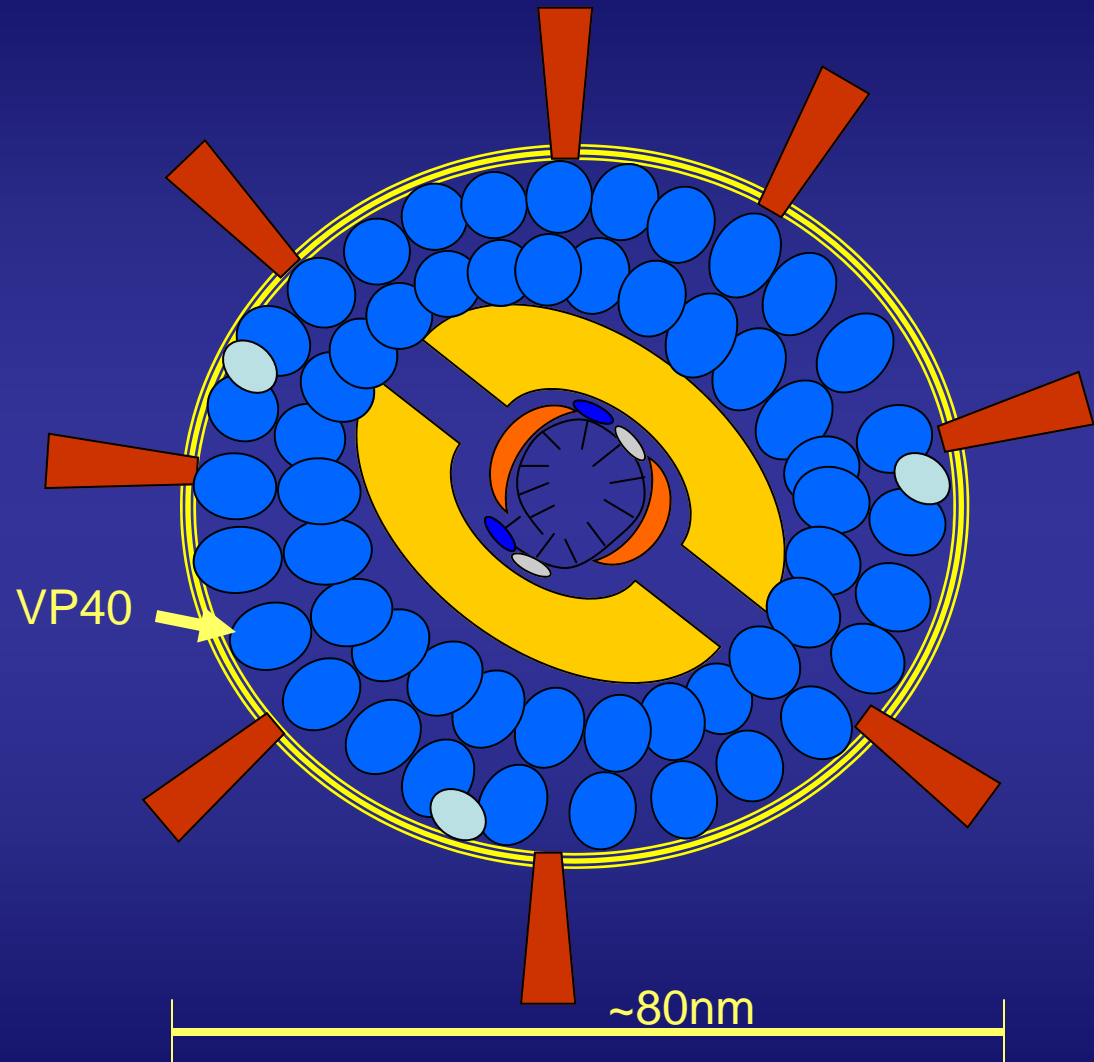
- Single strand negative sense RNA genome
- 7 genes, single segment ~19,000bp



# Filovirus Structure

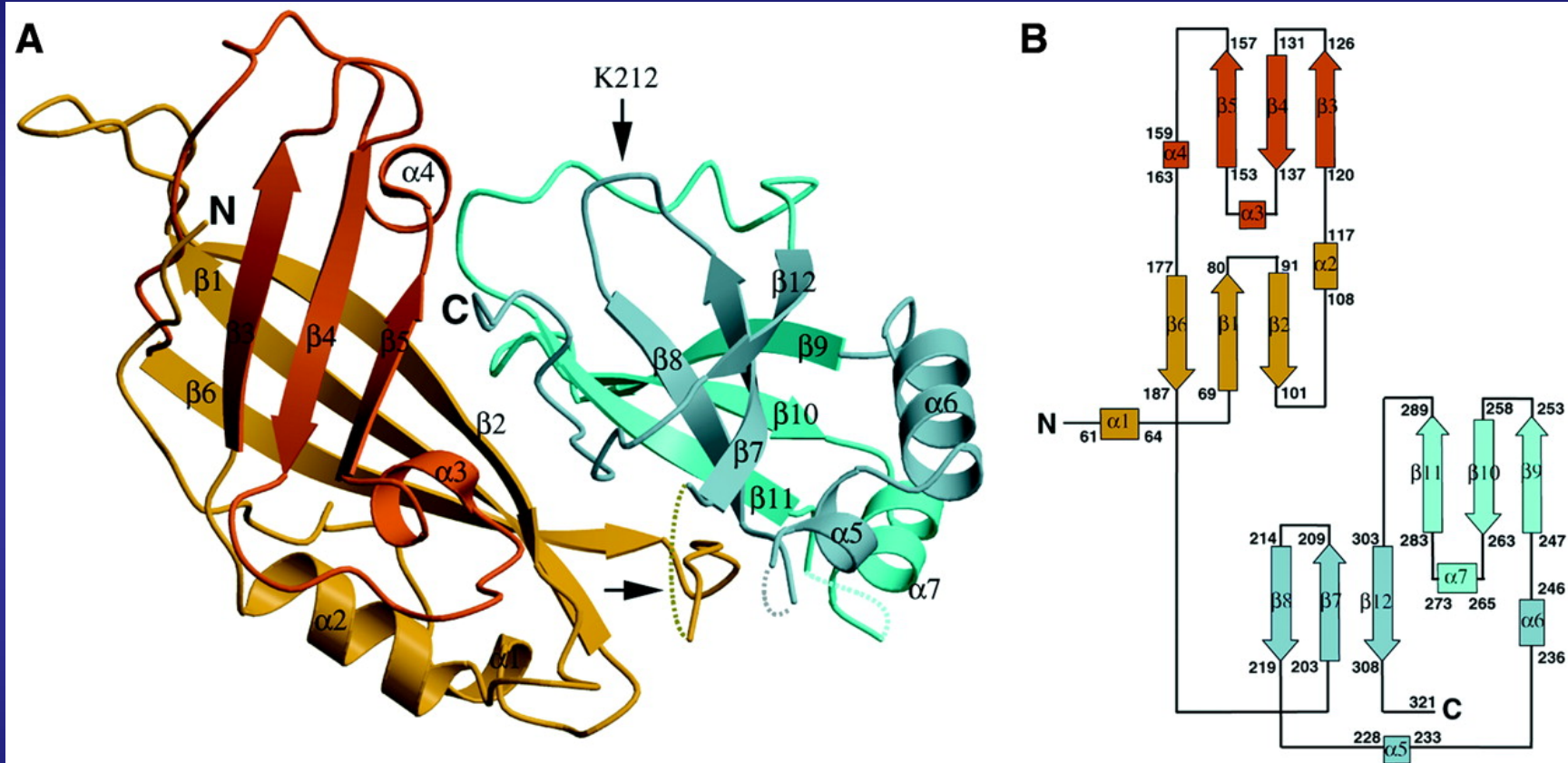


Longitudinal 2D  
Filamentous Particles



Transverse 2D section

# VP40-Matrix Protein



\*Exists in both monomeric and oligomeric conformations

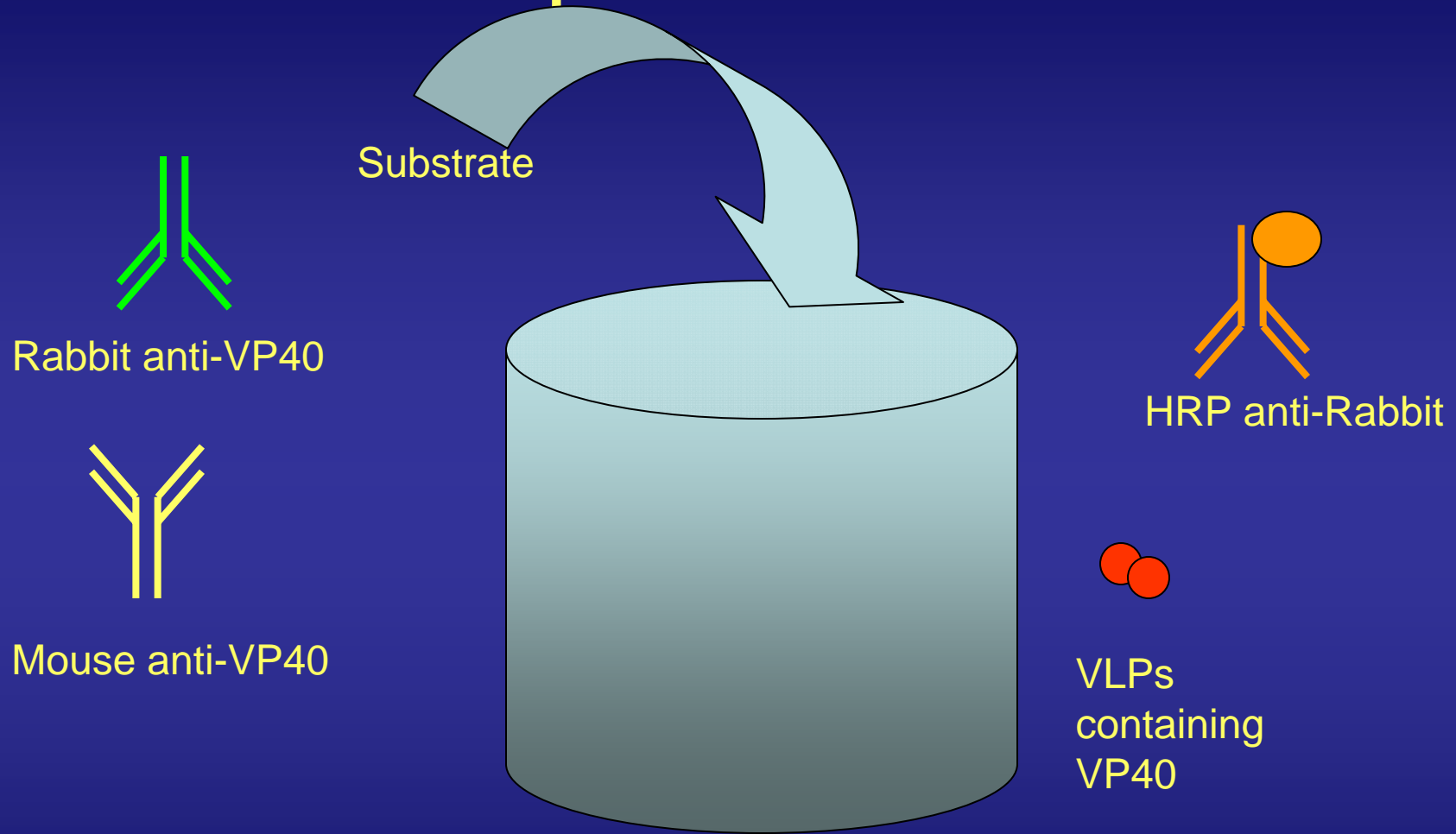
Dessen et al, 2000 EMBO Journal

# Objectives

- Develop an assay that measures Ebola virus / VLP release
- Study the effects of Ebola proteins on VLP production
- Determine the regions of VP40 that are important for viral release
- Screen for potential anti-viral therapeutic compounds able to inhibit viral release

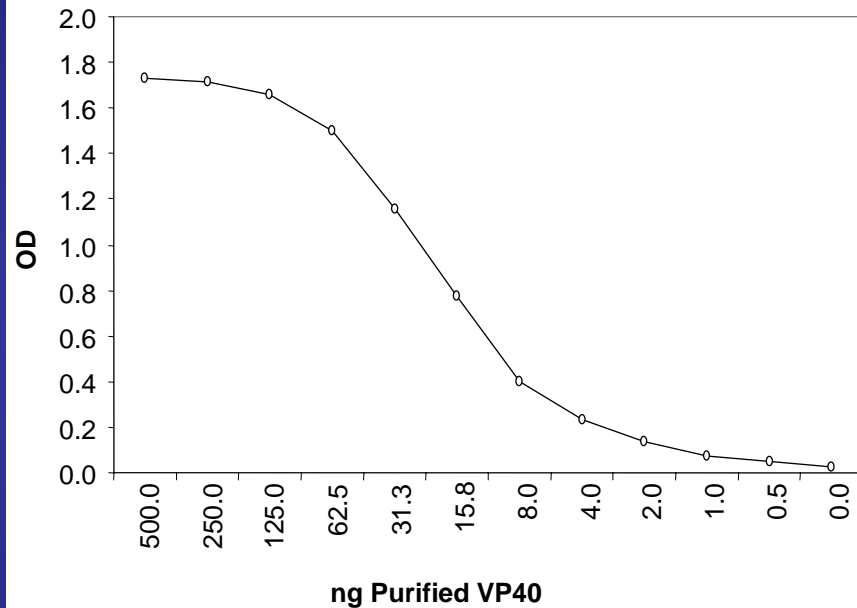


# Capture ELISA



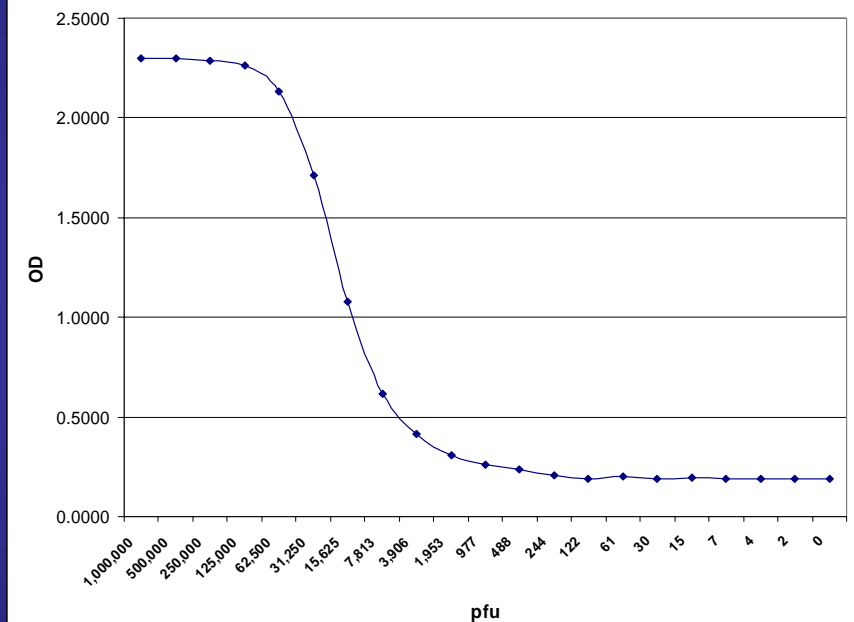
# Standardizing the assay

Purified VP40 protein



- Assay detects ~2ng of VP40 protein
- Linear from 10-125ng

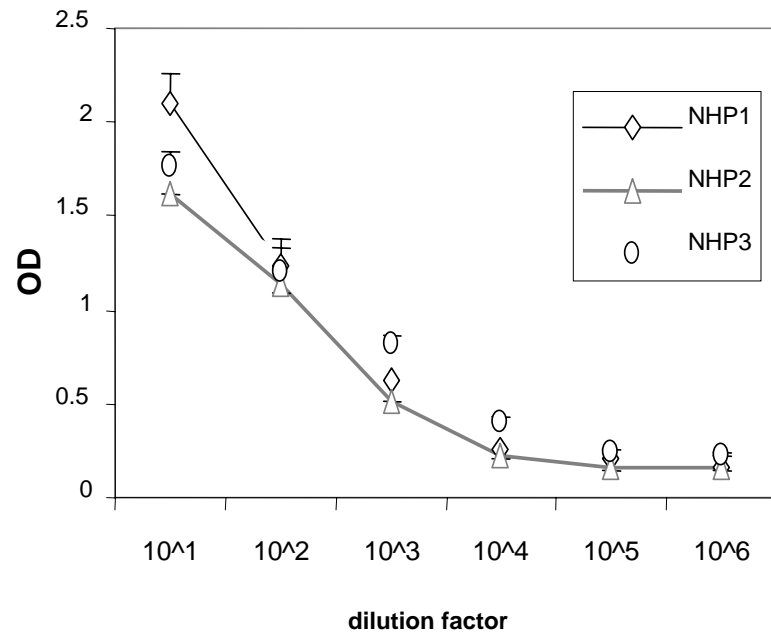
Inactivated Ebola virus



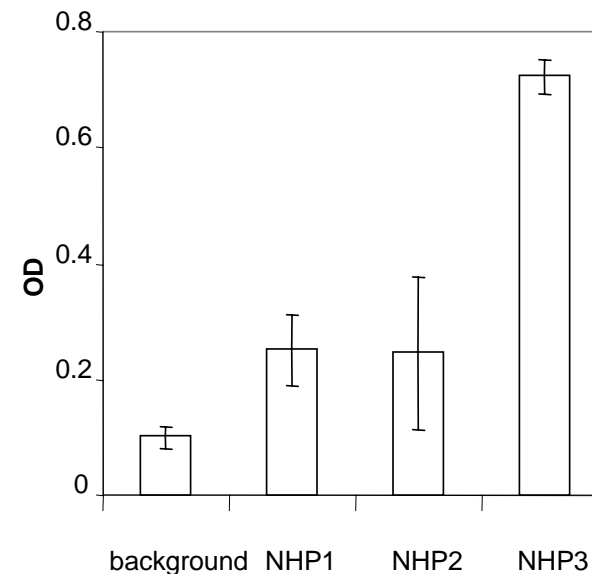
- Assay detects ~500pfu iEbola.
- Linear from 7000-100,000pfu

# Detecting Ebola virus in non-human primate samples

Dilutions of Liver Homogenate



Plasma samples

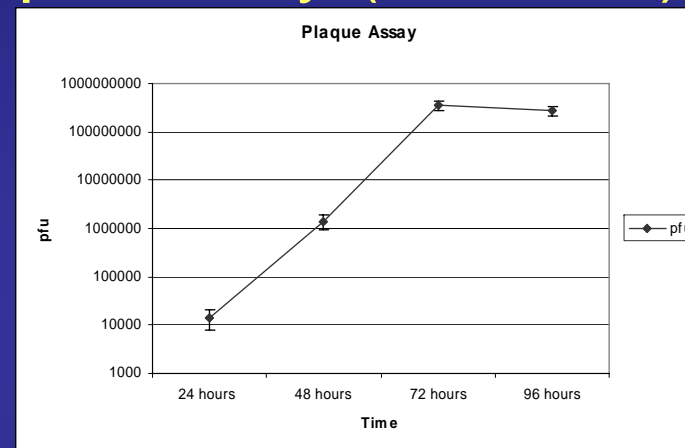
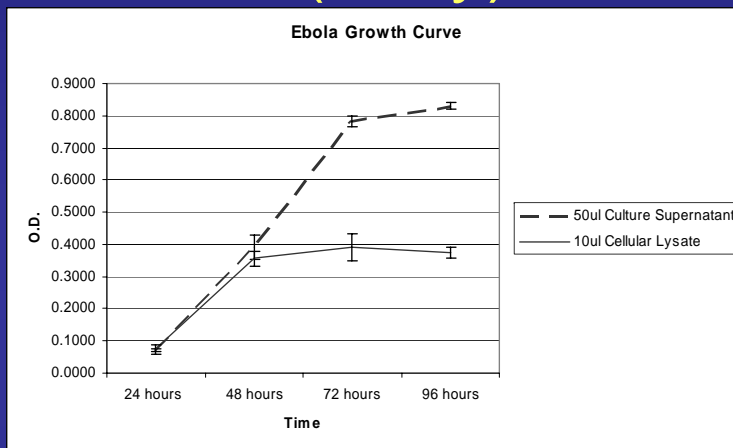


# Ebola virus growth curve

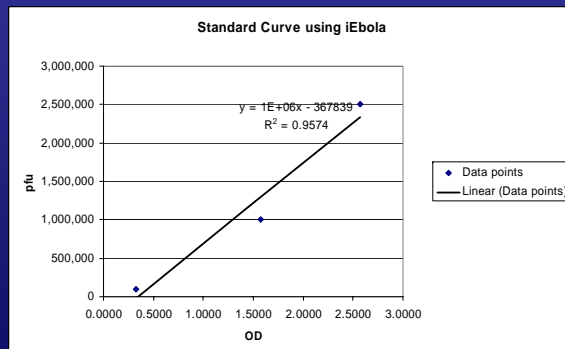
## ELISA / plaque assay

ELISA (1day)-BL2

Plaque assay (>1 week)-BL4



### Standard Curve



### Time Point

### Plaque Assay

### ELISA

48h

$1.2 \times 10^7$  pfu/ml

$1.0 \times 10^7$  pfu/ml

72h

$3.4 \times 10^8$  pfu/ml

$1.7 \times 10^8$  pfu/ml

96h

$2.7 \times 10^8$  pfu/ml

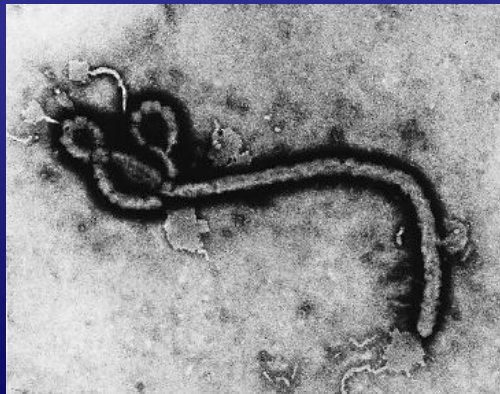
$2.4 \times 10^8$  pfu/ml

BL4 work, D. Swenson

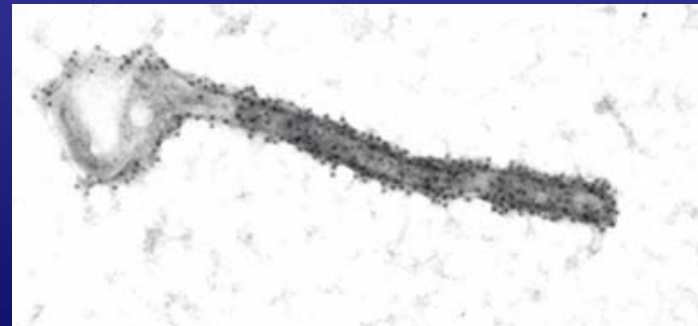
# VLP Structure, GP+VP40

- VP40 alone makes filamentous structures that resemble native virus.
- Adding GP increases the production of VLPs and makes the VLPs immunogenic.

**Ebola virus** (F. Murphy, CDC 1976)

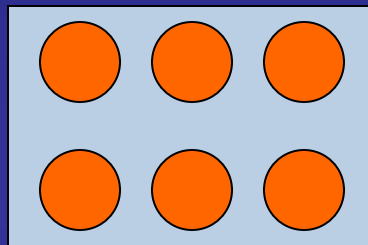


**GP+VP40 Virus Like Particle**



# Standard VLP assay conditions

Transfect  
VLP plasmids  
into 6 well plate  
@80% confluence

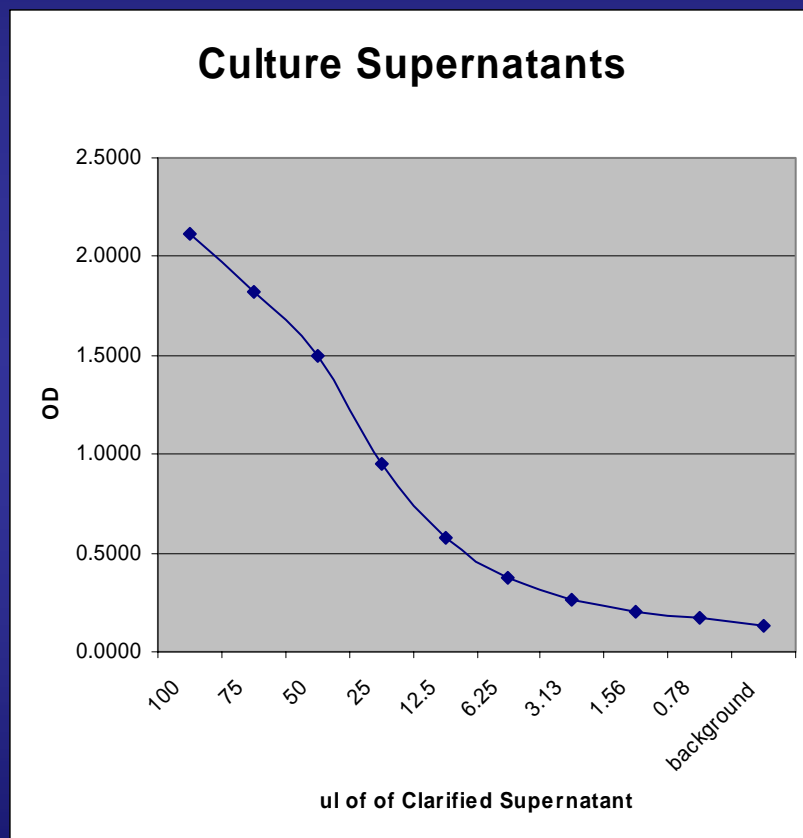


Incubate 48h

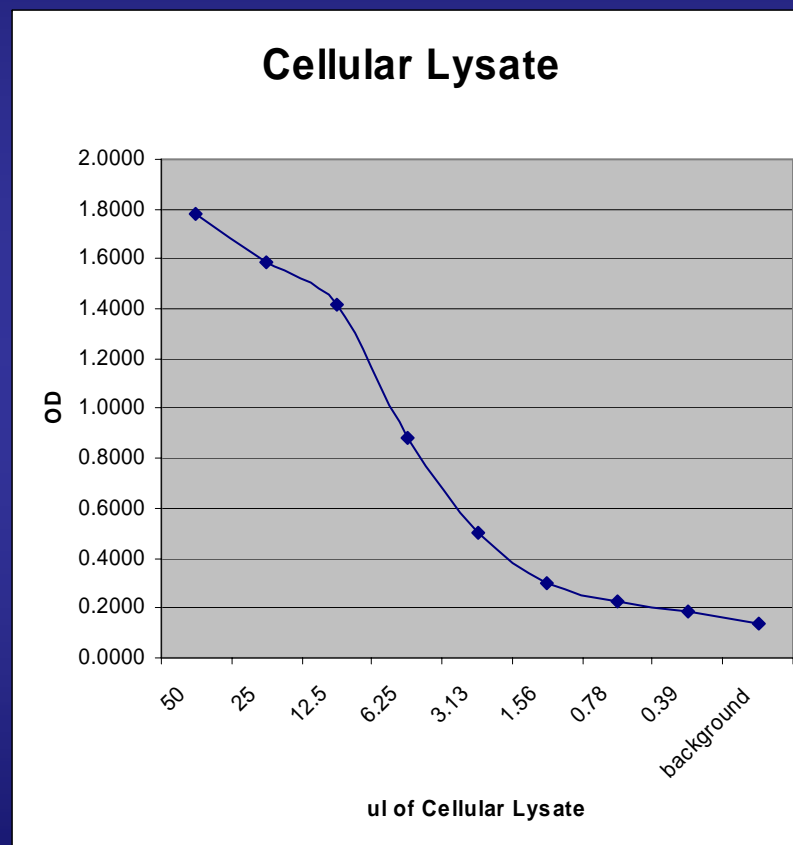
Harvest cell culture supernatant  
Wash the cells with PBS, harvest wash  
Harvest cell pellet and disrupt cells for cellular lysates

Perform ELISA

# Testing VLP Supernatants / Lysates

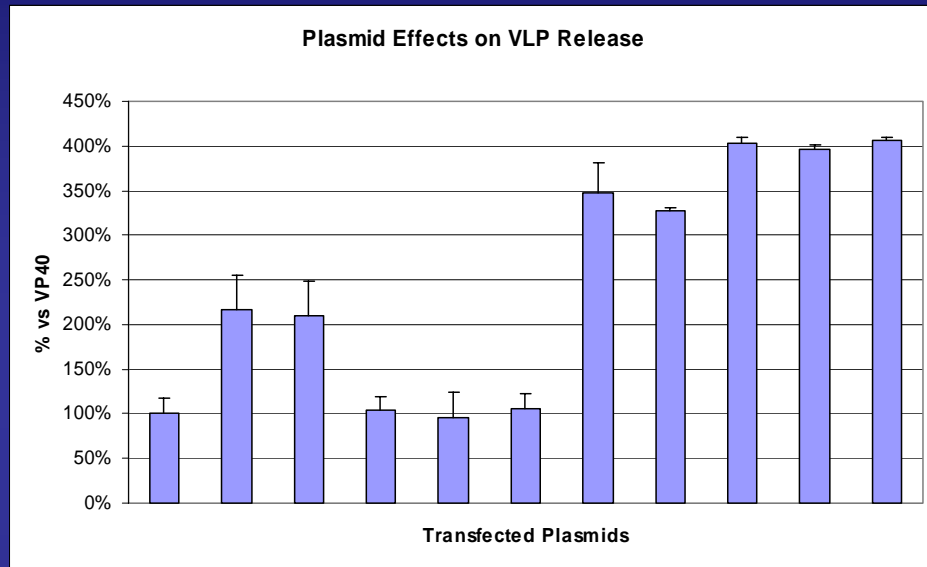


Can detect protein in 2ul of supernatant,  
linear range 12.5-75ul



Can detect protein in <1ul of lysate,  
linear range 3-25ul

# Effect of other plasmids on VLP formation



VP40	+	+	+	+	+	+	+	+	+	+	+
GP	-	+	-	-	-	-	+	+	+	+	+
NP	-	-	+	-	-	-	+	+	+	+	+
VP35	-	-	-	-	+	-	-	-	+	+	+
VP30	-	-	-	+	-	-	-	+	-	+	+
VP24	-	-	-	-	-	+	-	-	-	-	+

-GP and NP increase VP40 VLP release

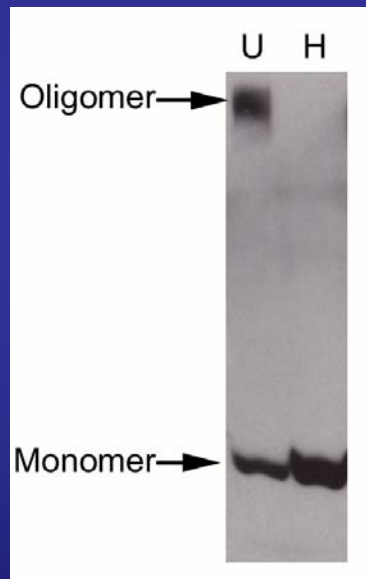
-VP30 slightly inhibits release in the presence of VP40 and NP

-VP35 promotes VLP release in the presence of VP40 and NP



# VP40 Oligomerization

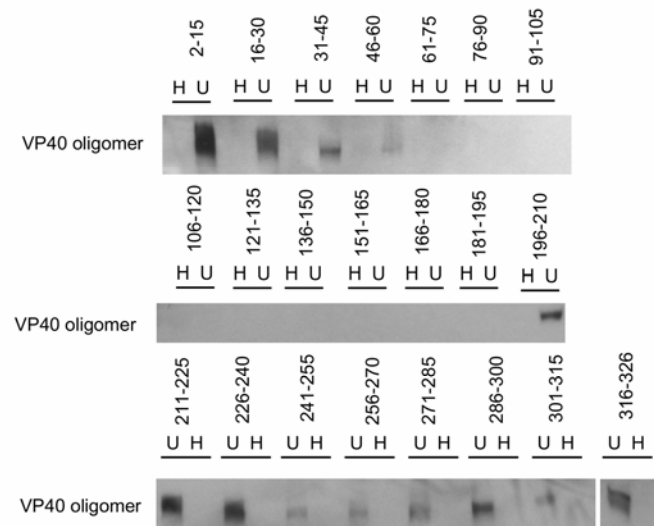
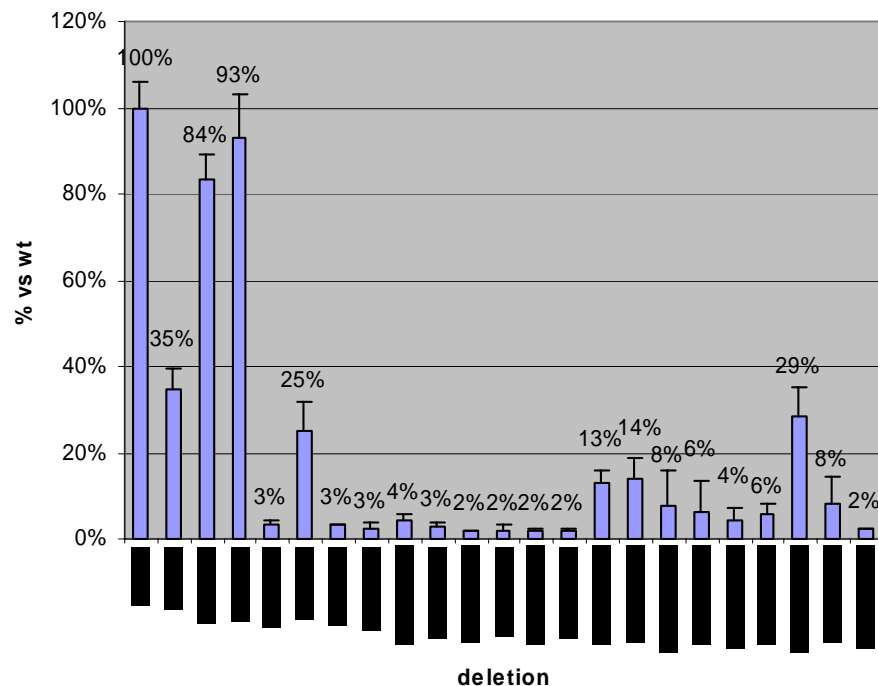
## Anti-VP40 Western



- VP40 exists in both monomeric and oligomeric forms.
- VP40 oligomers are SDS resistant, but heat sensitive.
- Oligomerization of VP40 is important for formation of VLPs.

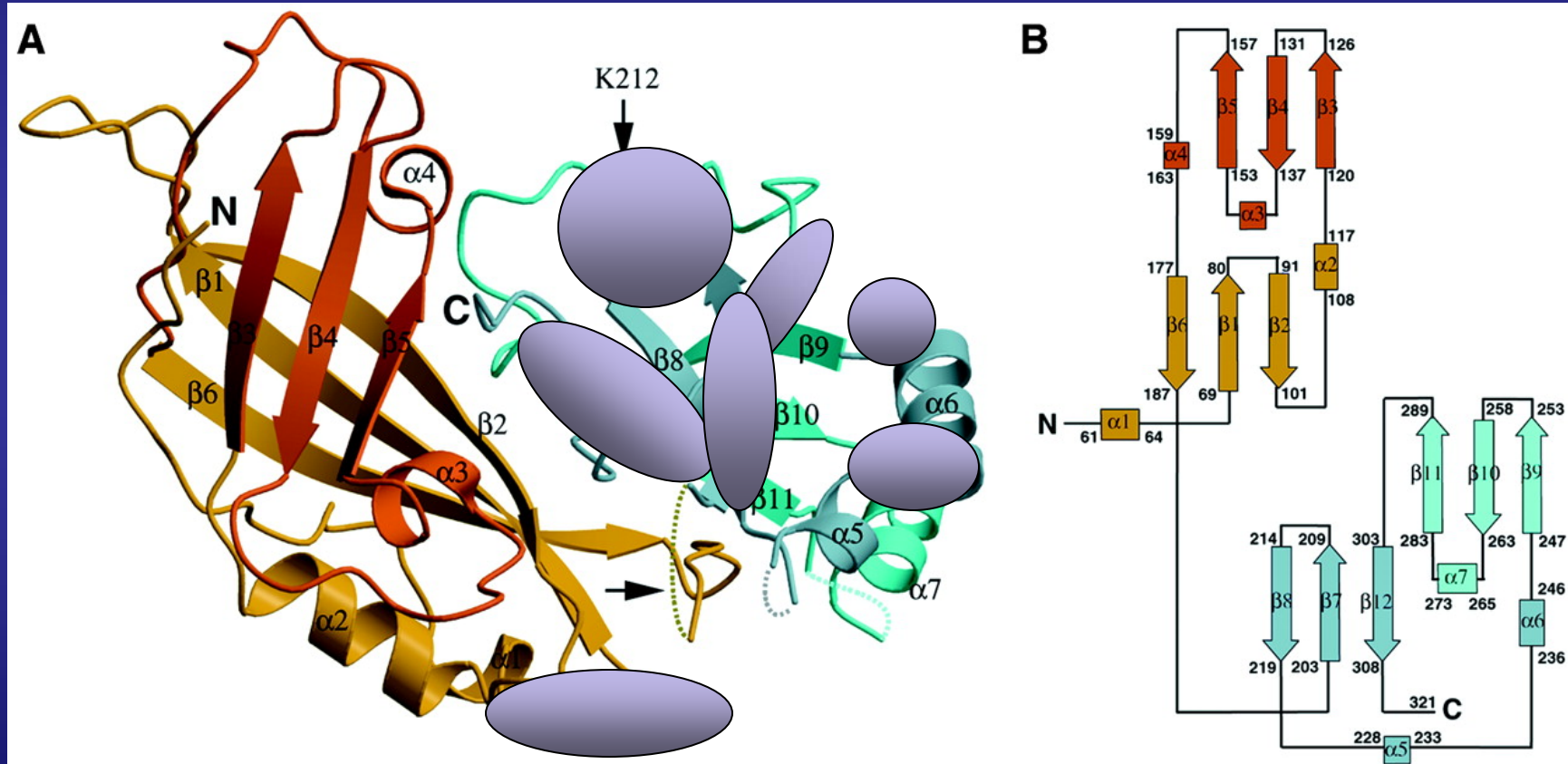
# VLP/oligomer formation

15amino acid deletions



T. Nelle constructed plasmids

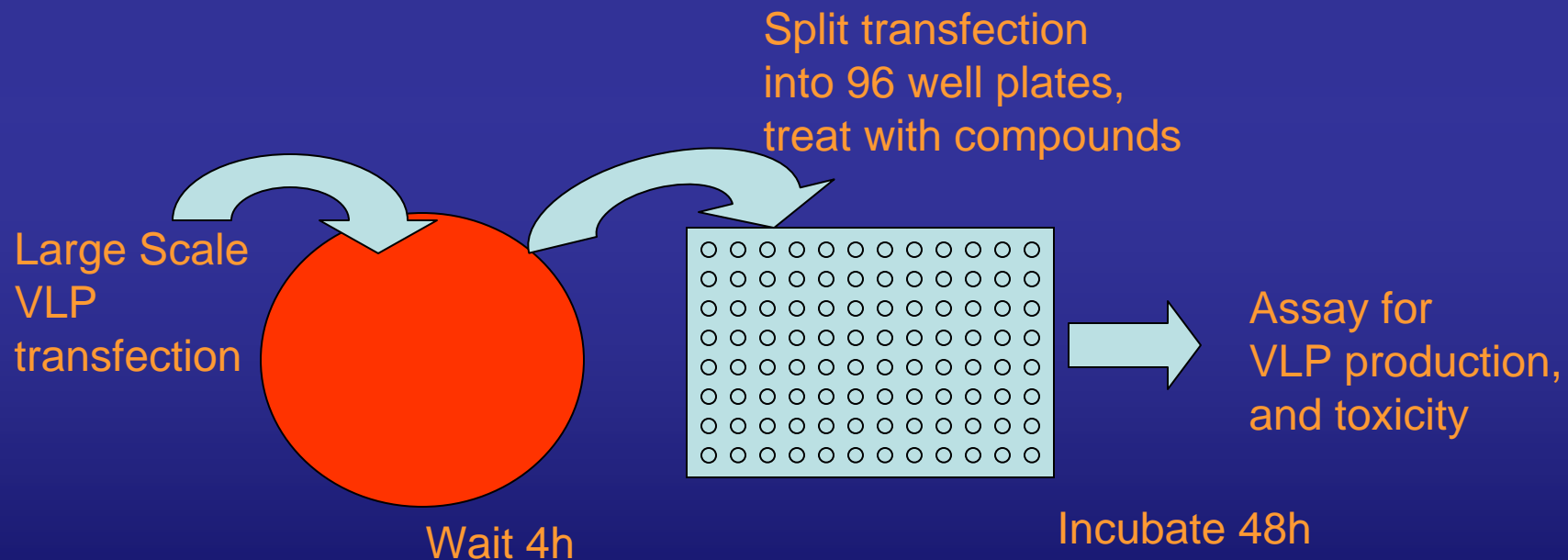
# Shaded deleted regions made oligomer / released VLPs



Dessen et al, 2000 EMBO Journal

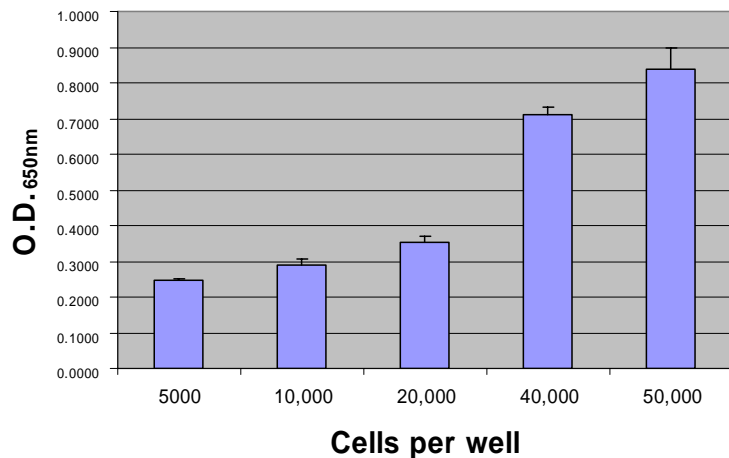
# ELISA can be utilized for therapeutic drug screening

- Identification of compounds that inhibit viral egress.



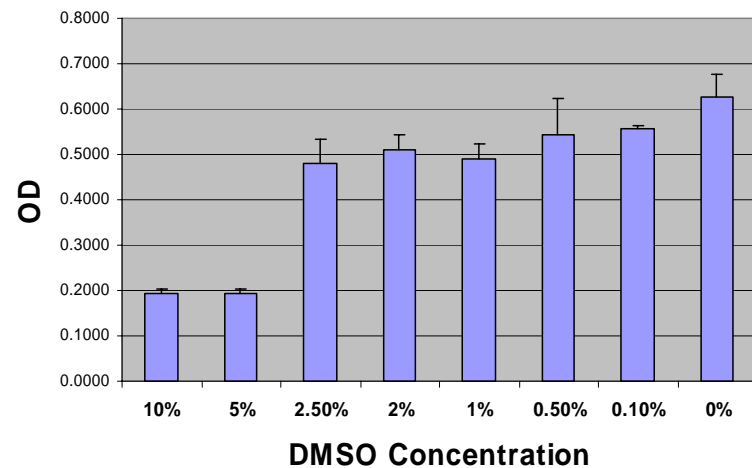
# Potential for therapeutic screening.

Sup from 96 well plate



40,000 cells / well is optimal for splitting/signal, at 50,000 wells are overgrown

VLP release 96 well DMSO treated



<2.5%DMSO did not affect VLP production or cell viability

# Accomplishments

- Developed and optimized ELISA that measures Ebola virus / VLP release
- Quantified the contribution of filoviral proteins on VLP formation
- Identified regions of VP40 that are critical for VLP formation release
- Prepared for antiviral therapeutic compound screening

# Summary

- We showed a sensitive ELISA for Ebola VLP / viral release
- Although not shown, the ELISA is useful for studying viral-host cell interactions
- Potentially useful as an alternative to plaque assays

# Acknowledgements

## Sina Bavari Lab

- Sina Bavari-leader of research group
- Javad Aman-research colleague
- Kelly Warfield & Dana Swenson-BSL4 experiments
- Gordon Ruthel-microscopy

## Other USAMRIID colleagues

- Shannon Mort-Undergraduate Student
- Sally Charles-Undergraduate Student
- Shirin Badie-Graduate Student
- Tim Nelle-constructed 15aa $\Delta$ -VP40 plasmids
- Other lab members and USAMRIID staff who support our research